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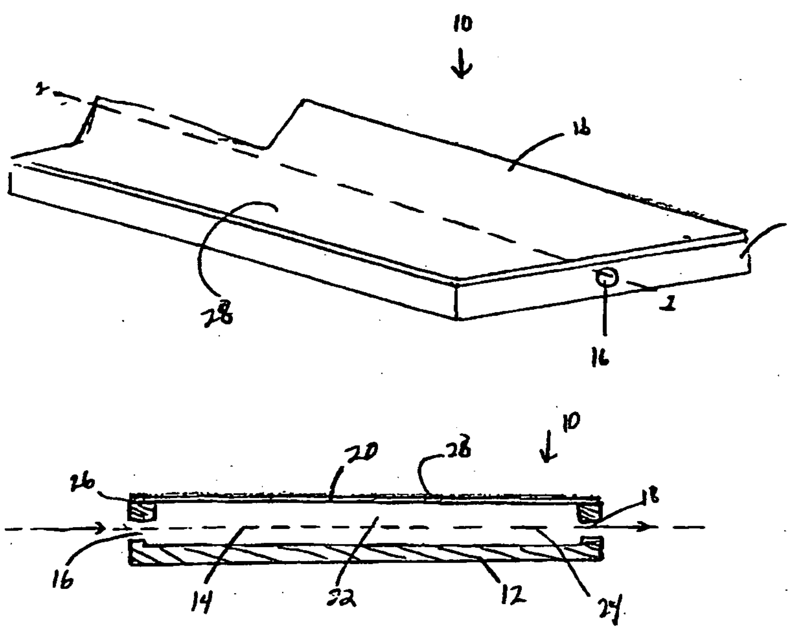

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(57) Abstract: A device for controlling the temperature of all or a portion of a microfluidic card is described. The device is comprised of a housing having a passageway formed therein which is enclosed by a heat-transfer film to define a closed channel. Fluid at a controlled temperature flows in the channel. The device is held in registration with a microfluidic card for thermal control of the entire card or of specific regions in the card. Methods for performing thermal cycling and temperature-responsive reactions using the device are also disclosed.

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MICROFLUIDIC CARD THERMAL CONTROL

INTRODUCTION

Field of the Invention

The present invention relates generally to a device for controlling the temperature in a microfluidic card. More particularly, the invention relates to a device for controlling the temperature in selected regions of a microfluidic card to provide for temperature control and/or thermal cycling during temperature responsive reactions in the card.

Background of the Invention

The use of microfluidic cards in the chemical and biochemical fields continues to expand. Applications of microfluidic cards include capillary electrophoresis, liquid chromatography, and chemical reaction and synthesis. In the biochemical field, microfluidic devices are used in the study of receptor-ligand interactions, enzyme-substrate interactions, cellular signaling pathways, and DNA amplification.

A microfluidic card is composed of a network of small capillary channels formed in a solid substrate. Various orifices in fluid communication with the channels serve as reservoirs and chambers for introducing reactants, reagents and test samples, and for mixing, holding, reacting, separating and/or detecting the reactants, reagents and products. The small volume of the microchannels and orifices offers considerable advantages over more conventionally-sized systems, including the need for less sample and reagents, rapid reaction times and efficient movement of components from one site to the next.

For many of the applications of use for microfluidic devices, thermal effects have a strong influence on the ensuing process. The temperature of the device or regions in the device can affect, among other things, sample stability, viscosity, chemical equilibria, pH and migration time of a given chemical species. In other applications of use, thermal cycling between two or more temperatures is desired. For example, polymerase chain reaction (PCR) amplification, nucleic acid hybridization, chemical labeling, nucleic acid fragmentation, transcription and sequencing require or benefit from precise control of reaction temperature.

The small size and small fluid volumes of microfluidic cards permits rapid and efficient heating and/or cooling, and a variety of approaches for controlling temperature and for thermal cycling of microfluidic cards have been proposed. For example, in U.S. Patent No. 5,641,400 (to Kaltenbach, *et al.*) heating or cooling of a miniaturized capillary electrophoresis device is provided by placing the card in thermal contact with a Peltier element.

In U.S. Patent No. 5,589,136 (to Northrup *et al.*), isolated thermal control in individual reaction chambers of a microfabricated silicon-based card is provided by directed contact of a wall of the chamber with a planar heater composed of a doped silicon for resistive heating.

In WO 97/12063, the temperature in a reaction chamber of a microfluidic device is controlled by means of a resistive heater deposited on the floor of the reaction chamber. The heater is connected to a power source for delivering a voltage across the resistive heater.

In WO 96/41864, a miniaturized microfabricated device is described, where the reaction chamber in the device is heated by means of external infrared or ultraviolet light sources.

In WO 98/50147 thermal control of a chemical reaction chamber is provided by contacting one or more walls of the chamber with a resistive heater in combination with a Peltier device.

There is still a need in the art, however, for a device and method which provides rapid, accurate control of the temperature of channels and orifices in microfluidic devices.

SUMMARY OF THE INVENTION

In one aspect, the invention includes a device for controlling the temperature of a microfluidic card or of selected regions, such as a reaction chamber, in a microfluidic card. In particular, the device is for use with a microfluidic card having features, such as reaction microchambers and microchannels, enclosed by a thin-film wall. The device comprises (i) a housing defining a passageway terminating at inlet and outlet ports; (ii) a heat-transfer film disposed on the housing enclosing the passageway to form a closed channel through which liquid can be directed between the inlet and outlet ports; and (iii) card registration elements for bringing the card in registration with the device. The heat-transfer film has one or more regions for heat transfer, wherein when the card is held in registration with the housing, the heat-transfer regions are in registry with regions of the thin-film wall of the card for transfer of heat across the wall and the film for controlling the temperature in those regions.

In one embodiment, the temperature control device includes one or more projections within the closed channel. In another embodiment, a first set of projections extends from the base of the housing, and a second set of projections extends from below the heat-transfer film. The two sets of projections are in an interdigitating arrangement to form a tortuous liquid flow path in the closed channel.

The projections, in some embodiments, are heat-insulating projections. For example, projections extending from below the heat-transfer film are positioned, in one embodiment, to reduce direct contact between the liquid in the closed channel and the heat-transfer film at regions other than the regions of heat transfer.

In another embodiment, the housing of the temperature control device has a base composed of a heat-transfer film, and the base has one or more regions for heat transfer. In this embodiment, the device can further include a heat-exchange device in contact with one or more of the heat-transfer regions in the base, for transfer of heat to or from the liquid across the base. In an alternative embodiment, the device is held in contact with a second microfluidic card having card features enclosed by a thin-film wall. When the second card is held in registration with the device, the heat-transfer regions in the base are in registry with the card features in the second card.

In yet another embodiment, the temperature control device includes a means for pressurizing liquid in the passageway.

In still another embodiment, the passageway in the device is cylindrical and has a base in a planar, opposing relationship with the heat-transfer film. The passageway includes a baffle which divides the passageway into two connecting chambers, with the inlet port entering one of the chambers in proximity to the base and the exit port in the other chamber in proximity to the base.

In another aspect, the invention includes a method for performing a temperature-responsive reaction in a microfluidic card having card features enclosed by a thin-film wall. The method includes contacting the card with the device as described above, with the heat transfer regions in heat-contact relationship with the card features, and introducing into the closed channel a liquid at a first controlled temperature to heat or cool the film to a selected temperature, thereby exposing reactants in the card to such temperature.

In one embodiment of this aspect, introducing the liquid includes achieving a pressure in the closed channel thereby causing the heat-transfer film to distend for intimate contact with the card.

In another embodiment, the liquid introduced into the closed channel is flushed with a second liquid at a second controlled temperature to expose the card contents to the second temperature. If desired, liquid at the first controlled temperature can be introduced again to the card.

In yet another aspect, the invention includes a method for performing a reaction requiring thermal cycling, such as polymerase chain reaction (PCR), in a microfluidic device, which may have a plurality of units for carrying out the reaction. The units will include reservoirs, channels and electrodes for transferring agents from one site to another. The method includes (i) contacting the microfluidic card with the temperature-control device described above, with the heat transfer regions in heat-contact relationship with the card features, *e.g.*, microchannels, reservoirs, etc., at which thermal cycling is desired; (ii) introducing into the closed channel a first liquid at a first controlled temperature to heat or cool the film to a selected temperature, thereby exposing reactants in the microchamber to such temperature; (iii) flushing the first liquid and any successive liquid in the closed channel with a second or any successive liquid at a second or successive controlled temperature to expose contents in the card to the second temperature; and (iv) repeating steps (ii) and (iii) at least one time. For the polymerase chain reaction, there will usually be three different temperatures for denaturation, primer annealing and extension.

The heat-transfer device can be compartmentalized, so as to have a plurality of streams flowing concurrently, where different regions of the card would be subjected to different thermal regimens.

These and other objects and features of the invention will be more fully appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a perspective view of a device in accordance with the invention for controlling the temperature in a microfluidic card;

Fig. 1B is a cross-sectional view along line 1-1 of Fig. 1A;

Figs. 2A-2B are fragmentary perspective views of an exemplary microfluidic card for use with the device of the invention, where the device is shown in assembled form (Fig. 2A) and in an exploded view (Fig. 2B);

Fig. 2C is a cross-sectional view along line 2-2 of Fig. 2A;

Figs. 3A-3B are fragmentary perspective views of another exemplary microfluidic card for use with the device of the invention, where the device is shown in assembled form (Fig. 3A) and in an exploded view (Fig. 3B);

Fig. 3C is a cross-sectional view along line 3-3 of Fig. 3A;

Fig. 4A is a sketch of a temperature-control device of the invention in operative position with a microfluidic device;

Fig. 4B is a cross-sectional view taken along line 4B-4B of Fig. 4A;

Fig. 4C is a cross-sectional view taken along line 4C-4C of Fig. 4A;

Fig. 5A-5B are sketches of a temperature-controlling device coming into operative position with a microfluidic device, where registration of the devices is assisted by guide pins (Fig. 5A) or by a detent (Fig. 5B);

Figs. 6A-6D show cross-sectional views of various embodiments of temperature-control device of the invention in operative position with a microfluidic card;

Fig. 7 is a cross-sectional view of a thermal-control device in contact with a microfluidic card, where the fluid flow path in the device provides for distinct regions of heat transfer;

Fig. 8 is a cross-sectional view of a microfluidic card held between opposing thermal-control devices;

Figs. 9A-9B show another embodiment of the invention where the temperature-control device is cylindrical, the cylindrical device shown in perspective view (Fig. 9A)and in cross-section (Fig. 9B);

Fig. 9C shows the device of Fig. 9A in operative position with a microfluidic card;

Fig. 10 shows an embodiment where the thermal-control device is an integral part of a

microfluidic card;

Figs. 11A-11B show in cross-sectional view embodiments of a temperature-control device of the invention connected to fluid supply lines for introducing fluid into the channel of the device, where in the embodiment of Fig. 11B, the device includes an external source to provide additional heat to the fluid; and

Fig. 12 shows a cross-section view of opposing temperature-control devices of the invention connected to fluid supply lines from reservoirs storing fluid at various temperatures for thermal cycling of a microfluidic card positioned between the temperature-control devices.

The drawings are diagrammatic only and not necessarily to scale and, particularly in some of the figures, the thicknesses of the devices and of the layers of which they are constructed, are much exaggerated for clarity of presentation.

DETAILED DESCRIPTION OF THE INVENTION

I. Temperature-Control Device

In one aspect, the invention includes a device for regulating the temperature of a microfluidic card. The device, depending on its geometry and configuration, is brought into contact with a microfluidic card for temperature regulation of the entire card or for regulation of selective regions, such as a microchamber, reservoir or channel, in the card.

A first embodiment of a temperature-control device in accordance with the invention is shown in Fig. 1A and in cross-section along line 1-1 in Fig. 1B. Device 10 is composed of a housing 12 in which a passageway 14 is formed. Passageway 14 terminates in inlet and outlet ports, indicated at 16, 18 in the drawings. Housing 12 is preferably formed from a material with poor thermal conductivity, *i.e.*, a heat-insulating material such as a plastic, and a variety of polymers and polymer blends are suitable, including, for example, polyolefins, polyolefin rubbers, rubbers, polyvinyl esters, acrylics, polyamides, and the like. Particularly preferred polymers are those amenable to a molding process, such as compression molding or injection molding, for fabrication of the housing and the passageway.

Disposed over the housing is a film 20 which encloses passageway 14 to form a channel 22 through which a fluid moves between the inlet and outlet ports. Film 20 provides for good thermal conductivity and heat transfer, being thin and/or composed of a material having good

thermal conductivity properties, *e.g.*, a material that permits a heat transfer by thermal conduction. Thermally-conductive materials include many metals, such as stainless steel, brass, copper, aluminum, gold, tin, lead, iron, zinc and silver, and metal alloys, including brass, bronze, aluminum alloys and copper alloys. Also suitable materials for film 20 are filled plastics, such as graphite-filled polypropylene or other metal-filled polymers. Film 20 is preferably thin, *e.g.*, in the range of 5-500 μm, more preferably between 10-125 μm. It will be appreciated that the thickness of film 20 is selected and varied according to the material from which it is made and the desired heat transfer properties. For example, a material having a lower thermal conductivity can be formed into a thin film to maximize heat transfer, whereas a material with high thermal conductivity could be formed into a slightly thicker film to achieve the desired heat transfer.

In one embodiment, which is described in more detail below, film 20 has some flexibility for movement away from the longitudinal axis of the device, represented by dashed line 24 in Fig. 1B, such that in profile the film or heat-transfer regions of the film are slightly convex with respect to the housing surface. The flexibility of the film allows for intimate contact between the film and a microfluidic card, as will be described.

Film 20 is joined to housing 12 by a suitable method, selected according to the housing and film materials and the desired strength of the seal therebetween. In the embodiment shown in Figs. 1A-1B, film 20 is joined at 26 about the periphery of housing 12 by, for example, a suitable adhesive or solder or by laser welding, heat sealing, or ultrasonic bonding.

Device 10 also includes one or more regions for heat transfer, which for the embodiment of the device shown in Figs. 1A-1B is taken as surface 28 of film 20. As will be described below, in use device 10 is placed in position with a microfluidic card for transfer of heat across film 20.

Turning now to Figs. 2A-2C, a fragment of an exemplary microfluidic card for use with the device of the invention is shown in perspective view in Fig.2A and in an exploded view in which the laminae of the card are separated in Fig. 2B. Card 30 comprises a first layer substrate 32 having sufficient thickness to accommodate the features, such as microchannels and microreservoirs, for operation of the card. Sealed to substrate 32 is a thin-film layer or cover 34. The thin-film cover when sealed to the substrate encloses microstructures formed in the substrate to form microchannels, microchambers, and the like. Perforations in the thin-film

cover provide for reservoirs or access ports for introduction or removal of components from the microstructures and/or introduction of electrodes. The thin-film cover is composed of any material suitable for bonding to the substrate of the card, with polymer films formed of acrylics and olefins being exemplary.

Device 30 includes card features 36a, 36b, which consist of microchannels 38a, 38b, formed in substrate 32. The substrate is usually of sufficient thickness to maintain structural integrity after the trenches or channels have been formed or may be supported by another film. This is important for configurations of the microchannel structures which are complex, or where there is a high density of trenches or channels. The width and depth of the microchannels are established by the dimensions of the channels formed in the substrate. The microchannels are typically of capillary dimension, with the larger cross-sectional dimension (usually the width) no greater than about 750 μm, more usually no greater than about 500 μm, and usually in the range of 50-250 μm. The smaller cross-sectional dimension (usually the depth) can be smaller than the other dimension, having a lower limit of about 10 μm.

Thin-film cover 34 is provided with perforations or holes, such as perforations 40a-40e in unit 36a. When the cover is sealed to the substrate, the perforations are positioned for alignment with the microreservoirs in the base to provide access ports to the reservoirs or other microstructures in the unit. Such reservoirs and access ports allow for introduction and removal of components and reactants, such as sample, buffer, solvents, enzymes, etc., to the reaction occurring in the microstructure unit. In some cases, a reservoir can be loaded with a reaction component during fabrication of the card, thus requiring no access port or corresponding perforation in the thin-film cover.

A cross-sectional view of unit 36a taken along line 2-2 of Fig. 2A is shown in Fig. 2C. Representative unit 36a is comprised of reservoirs 42, 44 in fluid communication via microchannel 46.

Not shown in Figs. 2A-2B, but which may be included in the microfluidic card are surface wires which extend into the reservoirs and which terminate in a contact electrode. The surface wires are connected to a controlled voltage source for providing an electric potential according to a predetermined regimen. The contact electrode and surface wires may be wires, electrically conductive paint or other means of electrical conduction. Potential differences across the electrodes are adjusted to electrokinetically move fluid about the microstructures.

Also not shown in the figures, but normally accompanying the card, is a detector for detection of the presence or absence of a species.

Another exemplary microfluidic card for use with the temperature-control device of the invention is shown in Figs. 3A-3C. With initial reference to Fig. 3A, which shows the card in assembled form, and in Fig. 3B, which shows an exploded view with the card laminae separated, card 50 is an elongate, flexible laminate comprised of a spacing lamina 51 sandwiched between a base 52 and a cover 53. Slits 54a, 54b, 54c having capillary cross-sectional dimensions are formed in spacing lamina 51. Cover lamina 53 includes holes, such as holes 55a, 55b, which provide access to the microstructures in the base when the lamina are in sealed contact and appropriately aligned.

In the embodiment of Figs. 3A-3C, the widths of the microchannels in the card are established by the widths of the slits formed in the spacing lamina, and the depth or thickness of the microchannels is determined by the thickness of the spacing layer. As seen best in the cross sectional view of Fig. 3C, which is a view taken along line 3-3 of Fig. 3A, microchannel 54b has a depth approximately corresponding to the thickness of spacing lamina 51. Reservoirs or access ports 56, 57 are provided when perforations 55b, 55a in cover 53 are aligned with the slit 54b in the spacing lamina. The depth of the reservoir, hence its volume, is established by the combined dimensions of the spacing lamina and cover layer.

Each lamina in the composite structure of Figs. 3A-3C is sealed to the surface of the opposing lamina by a sealing means appropriately selected according to the lamina materials. Typically, each of the lamina is a film, having sufficient structural integrity to hold the shape and dimensions of the microstructures. Preferred materials for the laminae include polymers, such as polyacrylics and polyolefins. The laminae are sealed using an adhesive, solder, heat seal or other means.

Manufacture of the exemplary microfluidic cards described in Figs. 2 and 3 is detailed in WO 99/19717, which is expressly incorporated herein by reference in its entirety.

It will be appreciated that the microfluidic cards shown in Figs. 2 and 3 are merely exemplary of such cards in general. The arrangement of microchannels, reservoirs, wells, and chambers in the card is readily varied according to the intended use. Various cards with a network of microchannels varying in complexity according to intended use have been described for example in U.S. Patent No. 5,587,128 (to Wilding, *et al.*) and WO 99/19717 (to Bjornson,

et al.), which is hereby incorporated by reference. The channels can be straight or in various patterns, such as serpentine or saw tooth, to allow for longer channels in a minimal space. The dimensions of the channels and chambers in microfluidic cards are typically in the range of 1-1000 μm, and preferably from about 2 μm to about 250 μm.

Figs. 4A-4C show various views of a microfluidic card, like that described in Figs. 3A-3C, in operative position with the thermal control device of the invention, as described in Figs. 1A-1B. In Fig. 4A, a fragmentary perspective view of a microfluidic card 60 composed of a spacing lamina 61 sandwiched between a base lamina 62 and a cover lamina 64. The laminae when assembled in sealing contact form and enclose the operative features (channels, reservoirs, chambers, etc) of the card. Card 60 is in contact with thermal-control device 66 comprised of a housing 68 and a heat-transfer film 70. The surface 72 of film 70 defines a heat-transfer region across which heat is transferred when the device is in contact with the microfluidic card.

Figs. 4B and 4C are cross-sectional views along line 4B-4B and 4C-4C of Fig. 4A, respectively. A passageway 74 in housing 68 enclosed by the heat-transfer film 70 defines a closed channel 76. The closed channel extends from an inlet port 78 and terminates at an outlet port, not visible in the figure.

In use, the thermal-control device is placed in operative position with the microfluidic card. By operative position it is meant that the microfluidic card is held in contact with the thermal-control device such that the thin-film wall of the card is in contact with the heat-transfer film of the thermal-control device. More specifically, the heat transfer region or regions of the device are in registry with one or more regions of the microfluidic card where temperature control is desired.

To assist in bringing and or/holding the card in registration with the thermal-control device, the device can include registration elements. Where the thermal-control device has width and/or length dimensions identical to the microfluidic card, the edges of the device serve as registration elements. Other possible registration elements are depicted in Figs. 5A-5B. In Fig. 5A, a microfluidic card 80 is brought into registration with a temperature-control device 82 by means of guide pins 84a, 84b, 84c which insert into depressions in the microfluidic card. In Fig. 5B, a microfluidic card 86 is brought into operative position with a thermal-control device 88 using detent 90, 92 to align the devices properly. In other embodiments, the thermal-control device and the microfluidic card differ in geometry and/or dimensions. Similar registration

elements and others can be used to bring the devices in alignment for heat transfer.

With continuing reference to Figs. 4A-4C, surface 72 of film 70 defines a region for transfer of heat across film 70 and base laminae 64, which is preferably a thin-film lamina, of the microfluidic card. As can be seen in viewing Fig. 4B, the thermal-control device of this embodiment provides for thermal control of reservoirs 94, 96 in the microfluidic card, as will as microchannel 98. A fluid introduced into channel 76 flows between the inlet and outlet ports. The fluid, depending on its temperature relative to the temperature of the contents in the microchannels and reservoirs of the microfluidic card, provides for heating or cooling of the card's contents.

A plastic card will have relatively poor thermal conductivity, which permits the maintenance of high temperature gradients within the plane of the card (i.e. zones of different temperatures). The thin cover film (and optionally thin body) of the plastic cards still allows good heat transfer in the vertical direction for rapid temperature variation.

In a preferred embodiment, the fluid introduced into the device is a liquid, and more preferably a liquid having a heat capacity equal to or greater than water, *e.g.*, a heat capacity of at least 1 Cal/g (4.18 J/g). Fluids capable of carrying an amount of thermal energy equal to or greater than water are readily known to those of skill in the art, and exemplary liquids include water, some glycols, oils and the like. These fluids can be used to more effectively heat or cool the device during operation. For electrokinetic operations such as electrophoresis, active cooling of the device enables the use of higher voltage gradients thereby generating more efficient movement or separations.

In other embodiments of the invention, the thermal-control device provides for temperature control of specific regions in a microfluidic card, and such thermal-control devices will now be discussed with reference to the remaining drawings.

A cross-sectional view of a thermal-control device in contact with a microfluidic card is shown in Fig. 6A. Thermal-control device 110 is comprised of housing member 112 and of a heat-transfer film 114 sealed to the housing member. Device 110 is in contact with a microfluidic card 116 comprised of a thin-film base lamina 118, a spacing lamina 119 and a cover lamina 120. Like the earlier described cards, card 116 includes access ports or reservoirs 122, 124 for introduction of samples and/or reagents. The reservoirs are in fluid communication via microchannel 126.

A passageway 130 formed in the housing member 112 of the thermal device 110 is enclosed by the heat-transfer film to form a closed channel 132 through which fluid for heat transfer flows. Extending into the closed channel is a projection 134. As can be seen in the figure, projection 134 is positioned to reduce contact between liquid flowing in the closed channel and defined regions of heat-transfer film 114. In this embodiment, projection 134 is positioned such that heat transfer across the heat-transfer film is reduced or blocked in the region of microchannel 126. Projection 134 could be hollow or could have a recessed top surface to further reduce thermal transfer or to allow passage of forced air to avoid heating certain areas of the card. Flanking each side of projection 134 is a heat region, indicated at 136, 138 in the figure, where temperature-controlled fluid in the closed channel directly contacts the heat transfer film for heat transfer between the liquid and the contents in reservoirs 122, 124, respectively.

Fig. 6B shows another embodiment, where a microfluidic card 140 is in contact with a thermal-control device 142. Device 142 includes a closed channel 144 which is designed to provide for heat transfer specifically with a microchannel 146 in the microfluidic card. Device 142 includes a projection 148 extending from housing 150. The projection is positioned such that when the temperature-control device is in operative position with the microfluidic card, a region of heat transfer 152 extends along the length of microchannel 146. Projections 154, 156 in housing 150 of the device direct the heat transfer medium away from reservoirs 158, 160 of the card, so that heat transfer between the reservoir contents and the heat transfer medium does not occur.

Another embodiment of the thermal-control device is illustrated in Fig. 6C. In this embodiment, device 170 is in registry with microfluidic card 172 comprised of a thin-film base lamina 173, a spacing lamina 174 and a cover lamina 175. Together the lamina when assembled define end reservoirs 176, 177 and a microchannel 178. Thin-film base lamina 173 is in heat-transfer contact with a heat-transfer film 179 sealed to a housing 180 of the temperature control device. Together the heat-transfer film and a passageway 181 formed in the housing define a closed channel 182 between an inlet port 183 and an outlet ports 184.

As can be seen in the figure, housing 180 includes projections 185a, 185b, 185c, 185d, which extend from the lower portion of the housing into the closed channel. Extending from the upper surface of housing 180, that is from the surface from which heat-transfer film 179 is

joined, are second projections 186a, 186b, 186c. The upper and lower projections are arranged in an interdigitating fashion to provide, in this embodiment, a tortuous fluid flow path. The tortuous flow path provides for mixing of the fluid in the closed channel, thereby reducing laminar flow and boundary layer effects, to achieve better temperature control of the contents in the microfluidic card.

Another embodiment of a temperature-control device is shown in cross-sectional view in Fig. 6D. The device is designed to control the temperature of specific reservoirs in microfluidic card 191 when in registration with the card. Card 191 includes a series of reservoir pairs, such as reservoirs 192a, 192b, in fluid communication via a microchannel, such as microchannel 193 connecting reservoirs 192a, 192b. The thermal-control device 190 is designed such that housing 194 includes a passageway 195 that together with a heat-transfer film 196 defines a closed channel for fluid flow between inlet and outlet ports. The passageway is constructed to provide for fluid contact with the heat-transfer film in card regions where heat transfer or control is desired. As seen in the figure, the fluid in the closed channel contacts the heat-transfer film at three heat-transfer regions, 197a, 197b, 197c, which correspond to one reservoir, such as reservoir 192b, in the reservoir pairs in the card.

The projections in the closed channel of the device shown in Fig. 6C may be composed of a heat-insulating material. In particular, the projections 198a, 198b, 198c, 198d which extend from the upper surface of the device housing are composed of a heat-insulating material to block or reduce heat transfer with the card in regions where heat control is not needed or desired. Such heat-insulation can be provided by construction of the projections with a polymer material, and in a preferred embodiment the projections are composed of the same material as that used for the device housing. Alternatively, the projections may be formed of a heat conducting material, so that there is good heat transfer between the projection in contact with the film and the film. In this instance, those areas of the film which are not in contact with the projections would be insulated from the liquid, so as to be relatively unaffected by changes in the temperature control liquid. One would have a heat insulating layer with the projections extending through the layer and in contact with the film. In this embodiment, the projections would be aligned with those areas in which heat transfer was desired.

The projections described with respect to Fig. 6C need not be formed of a heat-insulation material, as the function of the projections in this embodiment is to provide a tortuous fluid flow

path in combination with heat transfer across the span of the heat-transfer film in the region of the microchannel of the card.

Fig. 7 illustrates in cross-sectional view another embodiment of a thermal-control device. In this embodiment, the fluid flow path is directed by means of selectively positioned barriers in combination with heat-insulating projections, to define regions for heat transfer, as will now be described.

Thermal-control device 200 in Fig. 7, like those described above, is comprised of a housing 202 with a passageway therein. The housing is attached to a heat-transfer film 204, to define a closed channel 205. In direct contact with the heat-transfer film is a microfluidic card 206, comprised of a substrate 208 and a thin-film base 210. The card is designed to include features for operation, including end ports 212, 214, a reservoir 216 and a microchannel 218.

Positioned within closed channel 205 are projections 220a, 220b that extend from the lower surface of heat-transfer film 204. Disposed within the closed channel are barriers 222a, 222b for directing fluid entering at inlet port 224 along fluid flow path as indicated by the arrows in the figure and toward outlet port 226. Fluid flowing in the defined path comes into direct contact with the heat-transfer film at regions adjacent the end ports and the reservoir of the microfluidic card. The projections 220a, 220b in the closed channel block direct contact between the fluid and the region of the heat-transfer film adjacent the microchannel of the device. In this way, the temperature of the contents within the end ports and the reservoir can be varied, while the temperature in the microchannel is not varied.

Based on the foregoing, it will be appreciated that projections and barriers within the closed channel of the thermal-control device can be constructed and positioned to provide for or block heat transfer in any selected position in a microfluidic card of any design.

It will be appreciated that more than one thermal-control device can be used to regulate the temperature of a microfluid card. This embodiment is illustrated in Fig. 8, where a first thermal-control device 230 is in contact with one surface of a microfluidic card 232. A second thermal-control device 234 is in contact with the opposing surface of the microfluidic card. The microfluidic card is comprised of a substrate 236 having operational features constructed therein and enclosed by a first thin film wall 238 and a second thin-film wall 240. Together, the first and second thin-film walls define a first chamber 242 and a second chamber 244 in fluid communication via channel 246. An end port 248 is enclosed and defined by the first thin-film

wall, leaving the port open to the atmosphere for introduction of reagents and test samples as desired.

The first thermal-control device 230 in contact with the microfluidic card is comprised of a housing 250 and a heat-transfer film 252. A closed channel 254 provides for flow of a heat-transfer fluid between inlet and outlet ports 256, 258. Thermal-control device 230 provides for transfer of heat between the fluid in the channel and all of the opeational features of the microfluidic card.

The second thermal-control device 234 in contact with the upper thin-film wall of the microfluidic card is comprised of housing 260 and a heat-transfer film 262. Defined by the housing and the heat-transfer film is a closed channel 264 for fluid flow between inlet and outlet ports 266, 268. Projections 270a, 270b, 270c extend from the housing into the closed channel, and interdigitate with projections 272a, 272b which extend from the heat-transfer film 262. The arrangement of projections defines two regions for heat transfer, 274, 276. The regions for heat transfer allow for control of the temperature of the contents in chambers 242 and 244.

Figs. 9A-9C illustrate another embodiment of the invention, where the thermal-control device takes the form of a cylindrical body to provide temperature control in one or more specific regions of a microfluidic card. With initial reference to Figs. 9A-9B, a cylindrical temperature-control device 280 is shown in perspective view (Fig. 9A) and in cross-section (Fig. 9B). The device is formed of a housing 282 which defines a passageway 284 for fluid flow between an inlet port 286 and an outlet port 288, both ports positioned near the base of the device. Joined to one surface of the cylindrical housing is a heat-transfer film 290, which defines a planar surface 292 for transfer of heat across the film. The heat-transfer film is preferably a thin, flexible film formed of a material with good thermal conductivity, as discussed above.

As seen best in the cross-sectional view of the cylindrical device, the passageway is divided into two connecting chambers 294a, 294b, by a baffle 296. Fluid enters the device through the inlet port at the base of the device into chamber 294a. The fluid flows into and through the chamber, making direct contact with the heat-transfer film as it flows into the second chamber 294b. The fluid exits the second chamber via the outlet port, again positioned at the base of the device.

Such cylindrical temperature-control devices are shown in operative relationship with a

microfluidic card in Fig. 9C. A microfluidic card 300 comprised of a substrate 302 and a thin, laminate base for heat transfer 304 is shown. The card is as described above, and includes various microchannels, reservoirs, such as reservoir 306, and wells, such as wells 308, 310.

In direct contact with the laminate base of the microfluidic card are two cylindrical temperature-control devices 312, 314. The planar heat transfer surface 316 of device 312 is in registration with reservoir 306 in the device, to provide for temperature control of the fluid contents within the reservoir. Similarly, planar heat transfer surface 318 of device 314 is in registration with well 308 in the device, to provide for temperature control of the fluid contents within the well.

In an alternative embodiment, the temperature-control device of the invention is partially or entirely an integral part of the microfluidic card. An example of this embodiment is shown in Fig. 10, where a microfluidic card 320 is comprised of a substrate 322 having a series of chambers 324, 235, 326 formed in the housing and enclosed by a thin, laminate film 328. Upper surface 330 and lower surface 332 of card 320 are enclosed by a housing structure 334 which forms and defines a series of channels through which fluid flows. Inlet ports 336, 338 and outlet ports 340, 342 are provided in the housing for introducing a fluid at a controlled temperature. As can be seen, the housing is designed such that the fluid channels are in registration with the chambers in the microfluidic card, for temperature control of the chamber contents.

II. Method of Use

In another aspect, the invention includes a method for performing a temperature-responsive reaction in a microfluidic card of the type described above. The method will now be discussed with respect to Figs. 11A-11B.

Fig. 11A shows a cross-sectional view of a temperature-control device 350 attached to a fluid supply line 352. The device is comprised of a housing 354 having a passageway formed therein. The passageway is enclosed by a heat-transfer film 356 to define a closed channel 358 for fluid flow. Interdigitating projections 360, 362 extend into the channel and define a region 364 for heat transfer with a microfluidic card (not shown in the figure).

In use, a microfluidic card is brought into contact with the heat-transfer film of the temperature-control device in such a way that heat transfer region 364 is in registration with the

location on the card at which temperature control is desired. The card is brought into registration using registration elements described above with respect to Figs. 5A-5B and held in position by the elements or by another means, such as clips or clamps.

A fluid, *e.g.*, a liquid, is introduced into the closed channel via the fluid supply line connected to the inlet port of the device. The fluid supply line is joined to one or more feed lines which are in communication with a supply of fluid at a controlled temperature. As shown in Fig. 11A, fluid supply line 352 is connected via a two-way ball valve 368 to two feed lines 370, 372. Feed line 370 brings fluid from a supply reservoir (not shown in the figure) containing fluid at a first controlled temperature into the closed channel when the valve is appropriately positioned.

It will be appreciated that the flow rate of fluid through the closed channel can be adjusted by conventional methods. In one embodiment of the invention, the flow rate of fluid in the channel is adjusted to achieve a slight overpressure in the closed channel to cause the heat-transfer film of the device to distend outwardly, as depicted in phantom in Fig. 11A. The outward distension of the film achieves more intimate contact with the microfluidic card, as the film conforms to the shape of the microfluidic card to maximize the surface contact between the film and the card.

At a pre-selected time, the fluid in the channel at the first temperature is flushed from the channel by adjusting the valve to permit flow of fluid from feed line 372 into the supply line 352 and into the closed channel. Feed line 372 is connected to a supply reservoir of fluid at a second controlled temperature. Introduction of fluid at the second controlled temperature results in heat transfer across the heat-transfer film, to adjust the temperature of the contents in the microfluidic card to the second temperature.

It will be appreciated that repeated switching between the fluid at the first temperature and fluid at the second temperature can be done to repeatedly adjust the temperature of the microfluidic card. Suitable valving can provide the ability of switching between more than two different fluids, allowing cycling between multiple temperature points. There are a variety of applications which depend on temperature, including chemical and biochemical reactions. In exothermic reactions, the controlled temperature fluids can be at a temperature for removal of generated heat. In other reactions, the fluids maintain and/or control the temperature of the card contents. Exemplary reactions include enzymatic reactions, binding reactions, receptor-ligand

interactions and enzyme-substrate interactions.

Another application where control of temperature in reservoirs and channels of a microfluidic card is required is in loading the channels with a gel for capillary electrophoresis. Electrophoresis is typically performed in a gel that separates sample components as they migrate through the gel under the influence of an applied electric field. In capillary electrophoresis the gel is placed in the capillary-sized channel. Recently, thermoreversible gels for use in electrophoresis have been described (U.S. Patent No. 5,883,211). These gels undergo a phase change over a narrow temperature range, where above a defined temperature the viscosity of the gel lowers sufficiently for gel flow. Above the temperature, the viscosity increases such that the gel no longer flows.

The channels of a microfluidic card can be filled with a gel for capillary electrophoresis using the method described with respect to Fig. 11A. Fluid at a first temperature at or just above the melt temperature of the thermoreversible gel is introduced into the closed channel. The gel is introduced into sample ports of the card and allowed to flow into the channels, which are maintained at the desired temperature by the fluid in the thermal-control device. After loading, the gel is solidified by flushing the fluid at the first temperature with a fluid at a second, lower temperature that is below the phase change or melt temperature of the thermoreversible gel.

Fig. 11B illustrates a slight variation on the embodiment described in Fig. 11A, where a temperature control device 380 is formed of a housing 382 joined on upper and lower planar surfaces to heat transfer films 384, 386. Together the housing, having a passageway formed therein, and the heat transfer films define a closed channel 388 for flow of a heat transfer liquid. The upper heat transfer film 380 provides a planar surface for contact with a microfluidic card. The lower heat transfer film 386 has deposited on its surface external from the closed channel a device, such as a resistive heater 390, for heat exchange with the flowing liquid in the channel. The resistive heater is connected to a power source (not shown) via electrical connections 392 to provide a voltage across the heater. The heat generated is transferred across the heat-transfer film into the flowing liquid, providing for in-line heating of the fluid. A thermocouple or other feedback device can be placed in the channel to measure the temperature and provide for automatic control of the resistive heater.

In another aspect, the invention includes a method for performing a reaction in a

microcard where the reaction requires thermal cycling. This aspect of the invention will be discussed with reference to Fig. 12 and with respect to performing a polymerase chain reaction (PCR) amplification in a microfluidic card.

Fig. 12 shows a pair of opposing temperature-control devices 400, 402, each comprised of a housing 404, 406, having a passageway formed therein. Firmly sealed to each housing is a heat-transfer film, indicated at 408, 410 on housings 404, 402, respectively. The heat-transfer films enclose and define a closed channel in each device, indicated at 412, 414 in devices 400, 402 respectively. Opposing faces 416, 418 of heat-transfer films 408, 410 define a space 420 into which a microfluidic card is positioned during operation.

Devices 400, 402 are connected at inlet ports 422, 424 in each housing to a fluid supply line 426. The fluid supply line is connected through a 3-way ball valve 428 to fluid reservoirs 430, 432, 434. Each reservoir contains a liquid at a controlled temperature, indicated in the drawing as T1, T2 and T3. A pump 436 circulates fluid from the reservoirs through the temperature control devices.

The well-established PCR procedure requires repetition of heating and cooling cycles of a DNA target molecule in the presence of DNA primers, deoxynucleotide triphosphates and DNA polymerase enzymes. Each cycle of the reaction requires a brief heat treatment to separate the two strands of the DNA target molecule followed by a cooling step to anneal the primers to the DNA strands. A period of incubation allows for synthesis of DNA. The cycle is repeated for further amplification.

With respect to Fig. 12, a microfluidic card having the necessary structural units for carrying out a PCR reaction is placed between the temperature-control devices. The structural units in the card include reservoirs, channel and electrodes for transferring agents from one site to another. A sample is introduced into the reservoirs in the card, which serve as PCR reactors. The reservoirs are connected through channels to sources of nucleotides, which may include modified nucleotides, such as dideoxynucleotides, labeled nucleotides, *e.g.*, fluorescently-labeled nucleotides; enzymes, primers and any other reagents required for the amplification of the sample. The microfluidic card is positioned in registry with the temperature control device(s), where the regions of heat transfer are positioned to provide for heat transfer with the PCR reactor in each structural unit in the card. A DNA sample is introduced into the PCR reactor/reservoir in contact with the heat transfer film of the temperature control device. Using

electrokinesis, nucleotides, primer and enzymes are moved to the reactor from reservoir sources and the temperature is raised to about 90 °C to provide for denaturation by moving fluid from reservoir 430 into the closed channel of the temperature-control device(s). After sufficient time for denaturation to occur, the liquid flowing in the closed channel of the device is flushed with a liquid at 60 °C from reservoir 432 and the reactors are cooled to allow for copying of the ssDNA. These steps are repeated until the desired degree of amplification is achieved. During the process, additional amounts of the components or different components can be introduced into the reactor.

At the end of the PCR amplification, fluid from reservoir 434 at room temperature is introduced into the closed channel of the device, lowering the temperature of the microfluidic card for removal and analysis.

The subject invention provides for numerous advantages in the temperature control of small volumes, where rapid changes in temperature are desirable. A significant advantage of piping in liquid from external sources is that each source can contain a relatively large volume of previously thermally equilibrated fluid. Thus, a relatively simple thermal control scheme can be utilized. Also, thermal over-shoot or under-shoot is entirely avoided.

Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A device for controlling the temperature of the contents of at least one microstructure of a microfluidic card, wherein said microstructures are partially enclosed by a thin-film wall, comprising

a housing defining a passageway terminating at inlet and outlet ports;

a heat-transfer film disposed on said housing enclosing said passageway to form a closed channel through which liquid can be directed between the inlet and outlet ports; and

said heat-transfer film having one or more regions for heat transfer, wherein when said card is held in registration with said housing, said heat-transfer regions are in registry with said at least one microstructure of said card for transfer of heat across said wall and said film.

2. The device of claim 1, wherein the device further includes one or more projections within the closed channel.

3. The device of claim 2, wherein said housing has a base, said projections extend from said base, said device further comprising second projections extending from below said heat-transfer film, wherein said projections and said second projections are in an interdigitating arrangement to form a tortuous liquid flow path, where liquid flowing through said channel contacts said heat-transfer regions.

4. The device of claim 2, wherein the projections are heat-insulating projections.

5. The device of claim 4, wherein said projections extend from below said heat-transfer film and are positioned to reduce direct contact between said liquid in said closed channel and said heat-transfer film at regions other than said regions of heat transfer.

6. The device of claim 2, wherein the projections are heat-conducting projections.

7. The device of claim 1, wherein said housing has a base composed of a heat-transfer film, said base having one or more regions for heat transfer.

8. The device of claim 7, further including a heat-exchange device in contact with one or more of said heat-transfer regions in said base, for transfer of heat from said liquid across said base.

9. The device of claim 7, in combination with a second card having features enclosed by a thin-film wall, wherein when said card is held in registration with said housing, said heat-transfer regions are in registry with said at least one microstructure of said card for transfer of heat across said wall and said film.

10. The device of claim 1, further including means for pressurizing liquid in the passageway.

11. The device of claim 1, wherein said passageway is cylindrical and has a base in a planar, opposing relationship with the heat-transfer film, said passageway including a baffle which divides the passageway into two connecting chambers, with the inlet port entering one of the chambers in proximity to the base and the exit port in the other chamber in proximity to the base.

12. A device for controlling the temperature of the contents in a plurality of microstructures in a microfluidic card, the plurality of microstructures in the card partially enclosed by a thin-film lamina, comprising

a housing defining a passageway terminating at inlet and outlet ports;

a heat-transfer film disposed on said housing enclosing said passageway to form a closed channel through which liquid can be directed between the inlet and outlet ports; and

registration elements for bringing said card in registration with said housing,

said heat-transfer film having a plurality of regions for heat transfer, wherein when said card is held in registration with said housing, said heat-transfer regions are in registry with said plurality of microstructures in said card for transfer of heat across said lamina and said film, thereby providing temperature control to each microstructure in said plurality.

13. A method for performing a temperature-responsive reaction in a microfluidic card having features enclosed by a thin-film wall, comprising

contacting the card with the device according to claim 1, with said heat transfer regions in heat-contact relationship with said card features,

introducing into the closed channel a liquid at a first controlled temperature to heat or cool said film to a selected temperature, thereby exposing reactants in the card to such temperature.

14. The method of claim 13. wherein said introducing comprises achieving a pressure in the closed channel thereby causing the heat-transfer film to distend for intimate contact with the card.

15. The method of claim 13, which further includes the step of flushing the liquid, introduced into the closed channel with a second liquid at a second controlled temperature to expose contents in the card to the second temperature.

16. The method of claim 15, which further includes the step of repeating said introducing step.

17. A method for performing temperature-responsive reactions in a plurality of microstructures in a microfluidic card having such microstructures partially enclosed by a thin-film lamina, comprising

contacting the card with the device comprised of (i) a housing defining a passageway terminating at inlet and outlet ports; (ii) a heat-transfer film disposed on said housing enclosing said passageway to form a closed channel through which liquid can be directed between the inlet and outlet ports; and (iii) registration elements for bringing said card in registration with said housing, where said heat-transfer film includes a plurality of regions for heat transfer, and when said card is held in registration with said housing, said heat-transfer regions are in registry with said plurality of microstructures in said card for transfer of heat across said lamina and said film,

introducing into the closed channel a liquid at a first controlled temperature to heat or

cool said film to a selected temperature, thereby exposing reactants in each of said plurality of microstructures in the card to such temperature.

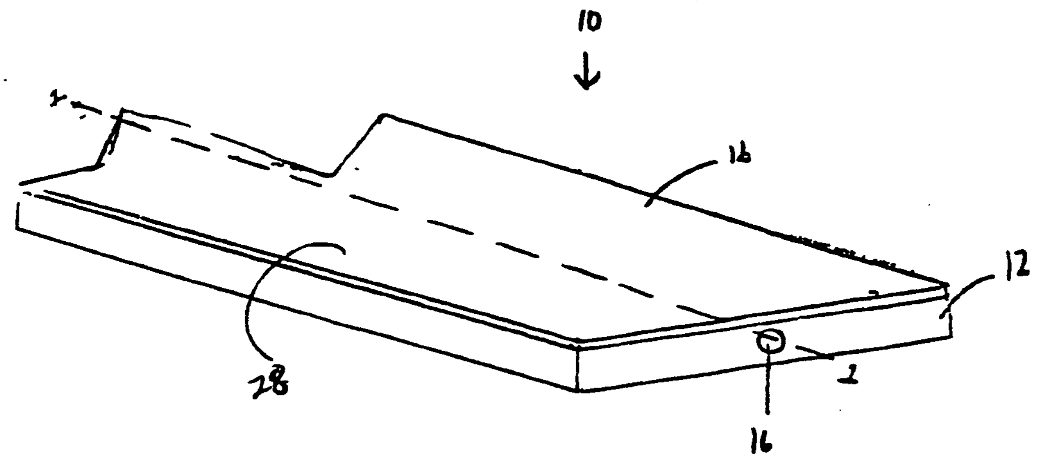
18. A method for performing a reaction requiring thermal cycling in a microfluidic a card having microstructures partially enclosed by a thin-film lamina, comprising

(i) contacting the card with the device according to claim 1, with said heat transfer regions in heat-contact relationship with said card microstructures,

(ii) introducing into the closed channel a first liquid at a first controlled temperature to heat or cool said film to a selected temperature, thereby exposing reactants in the microstructures to such temperature,

(iii) flushing the first liquid in said closed channel with one or more successive liquids at different controlled temperatures to expose contents in the microstructures to the different temperatures; and

(iv) repeating steps (ii) and (iii) at least once.



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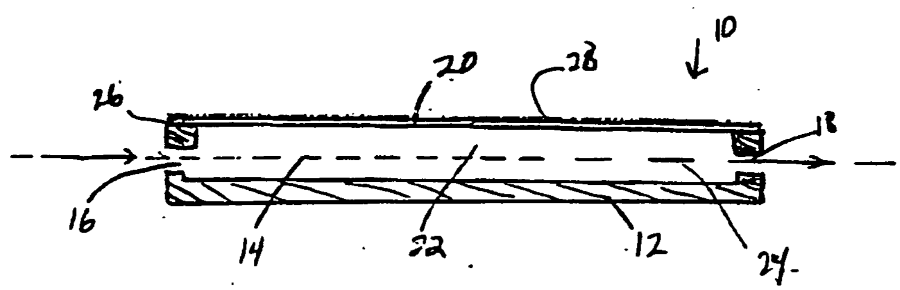

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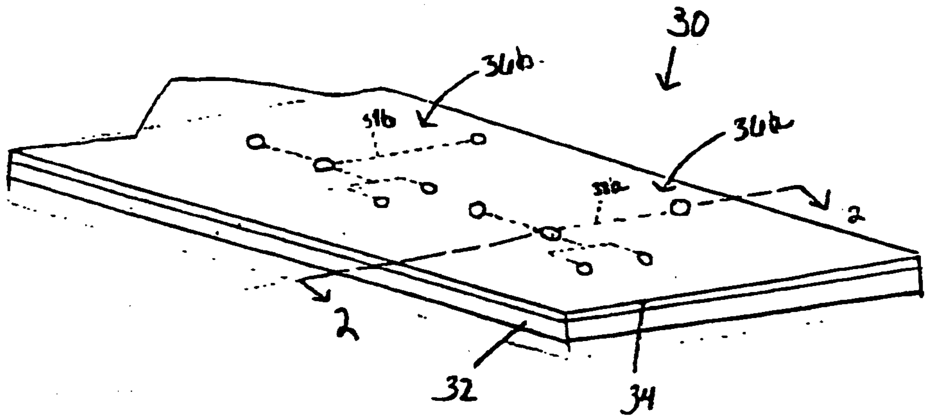

Fig. 2A

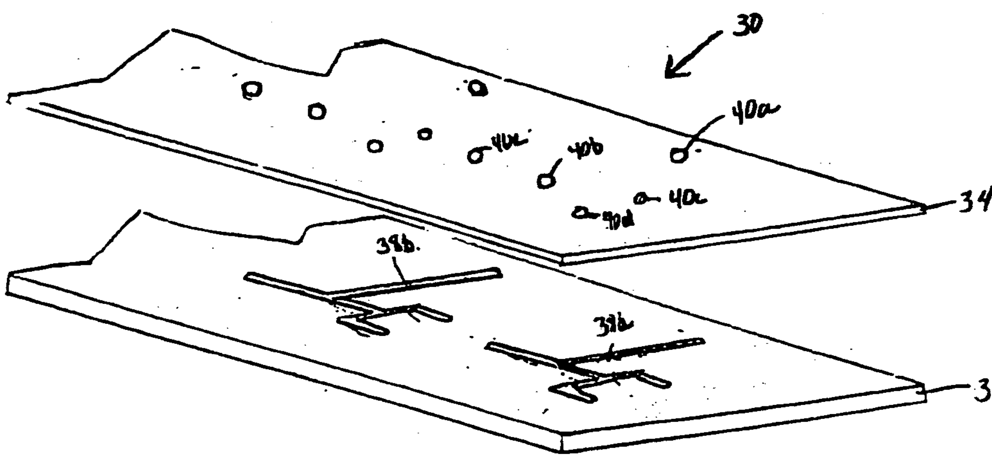

Fig. 2B

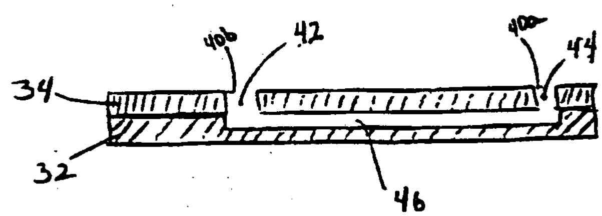

Fig. 2C

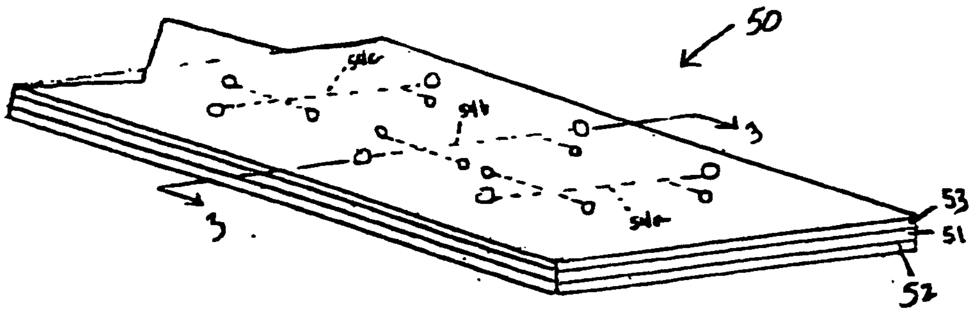

Fig. 3A

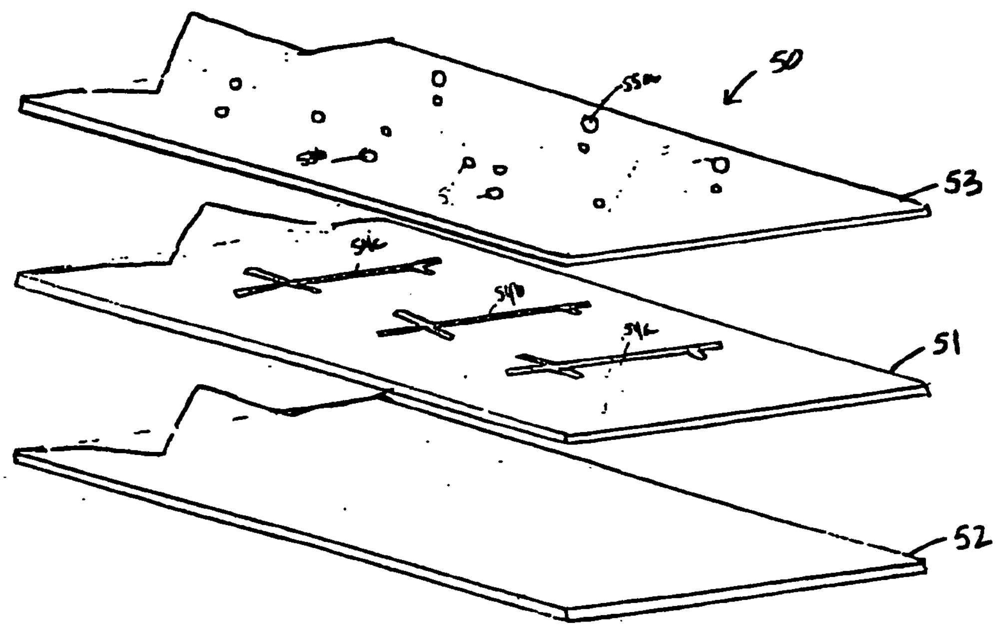

Fig. 3B

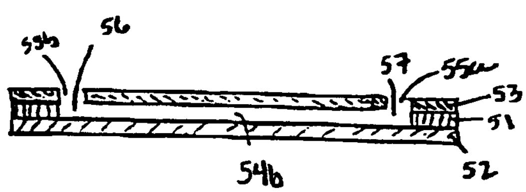

Fig. 3C

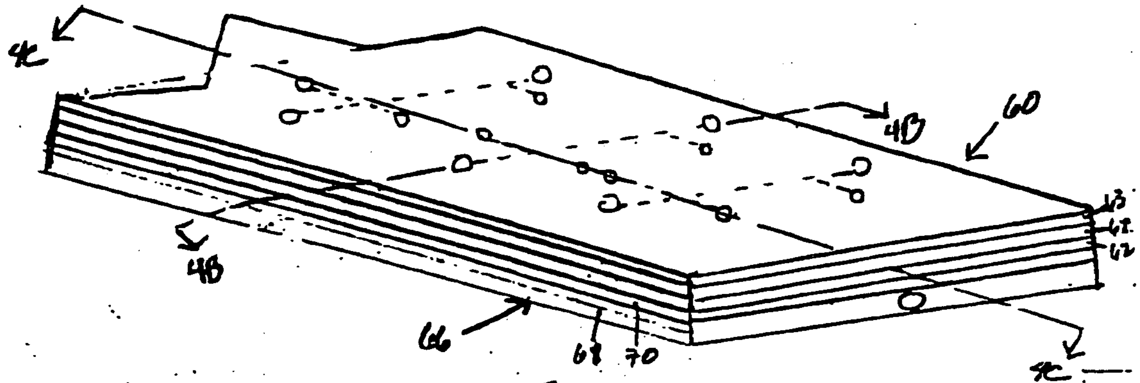
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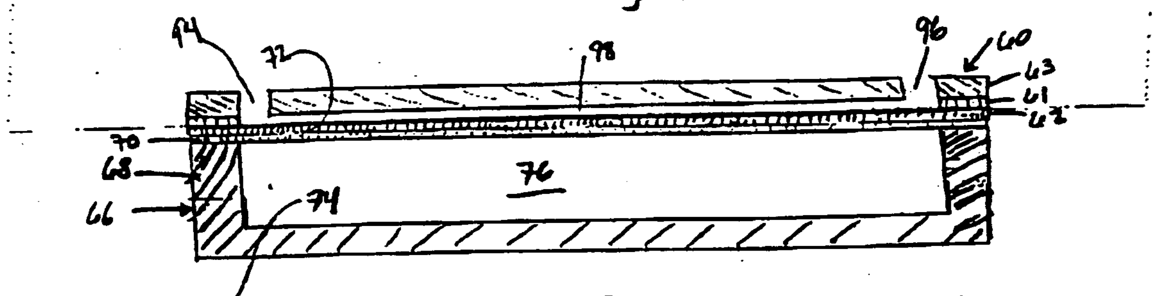
Fig. 4A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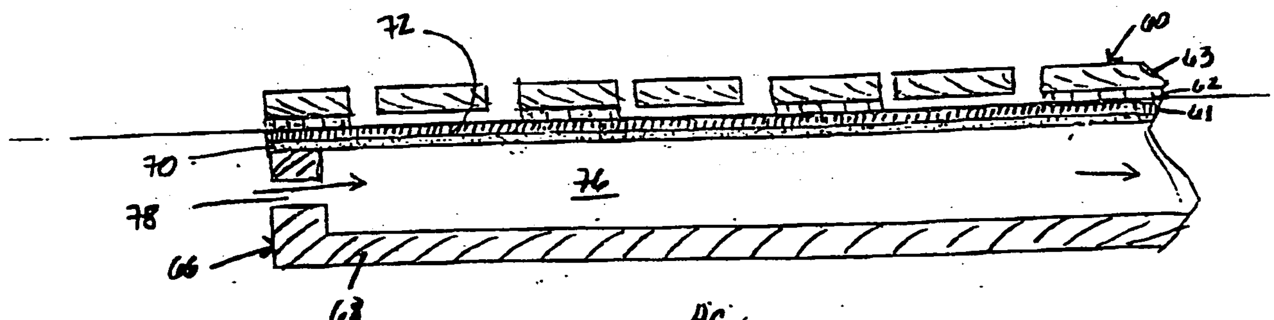
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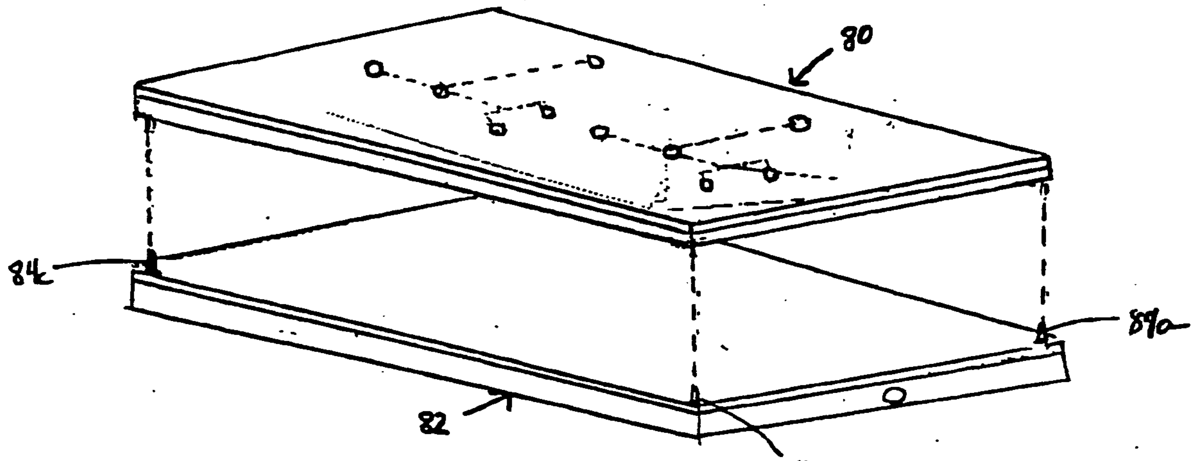

Fig. 5A

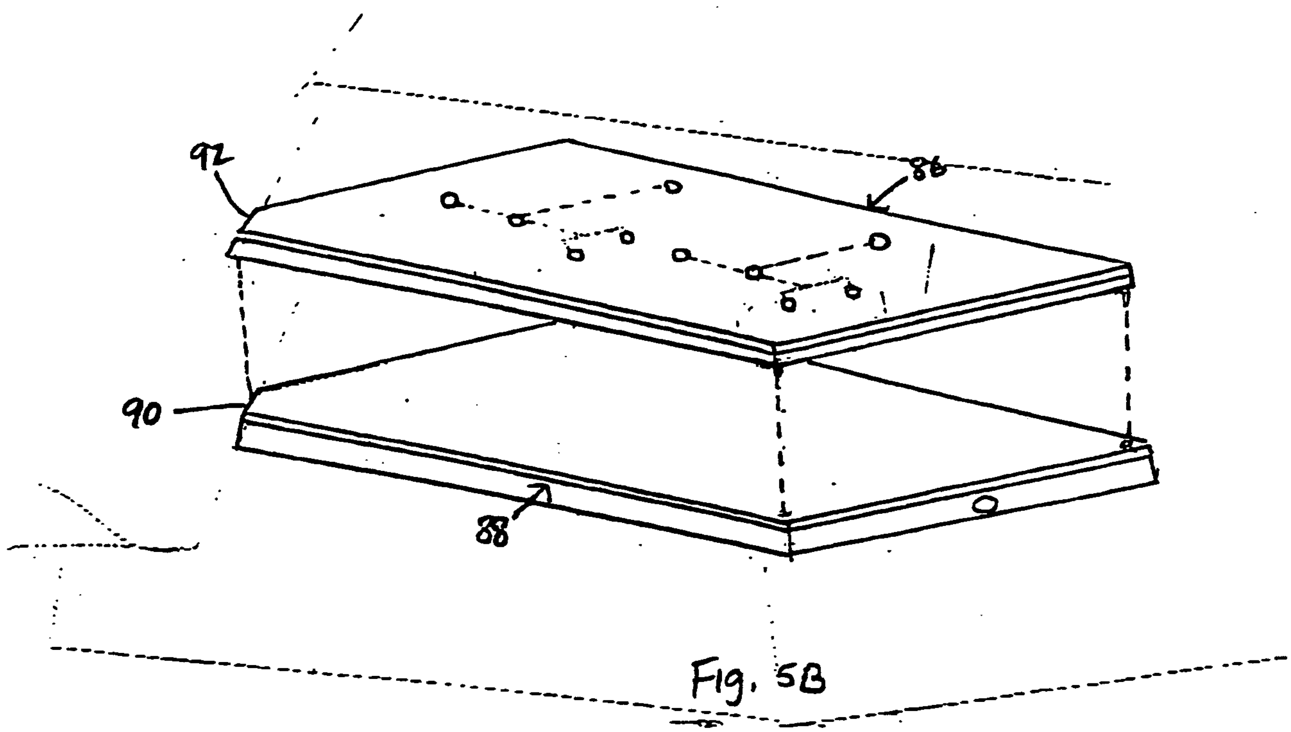

Fig. 5B

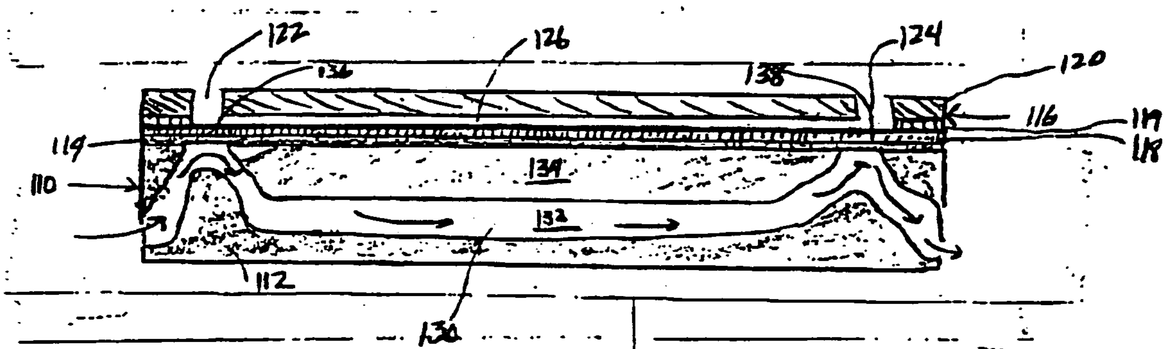
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Fig. 6A

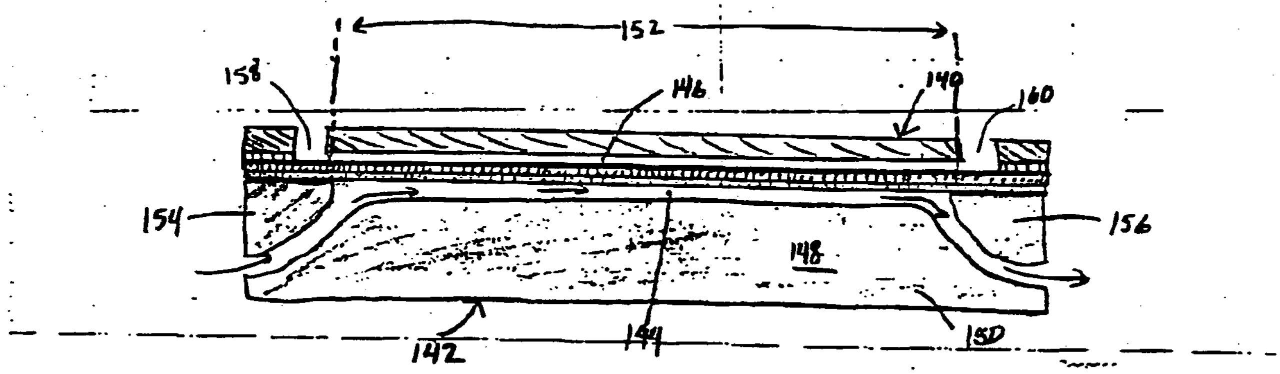
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Fig. 6B

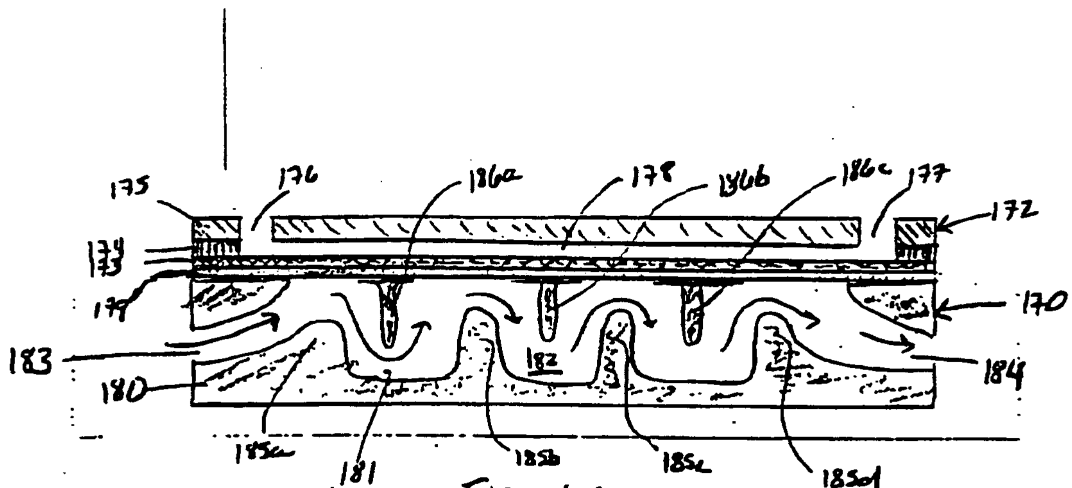
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Fig. 6C

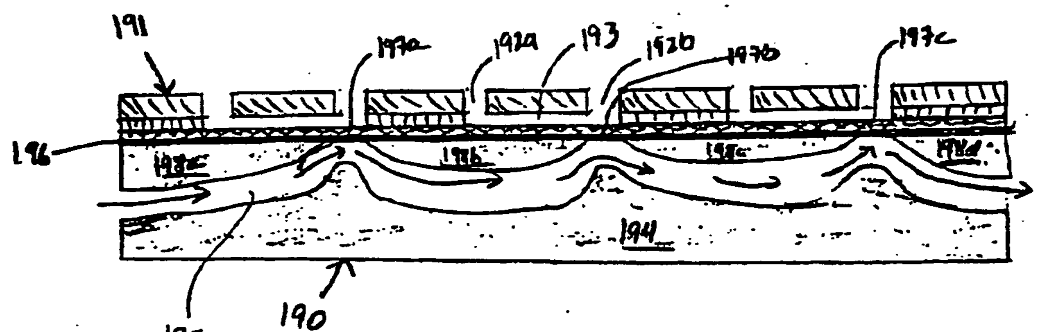
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Fig. 6D

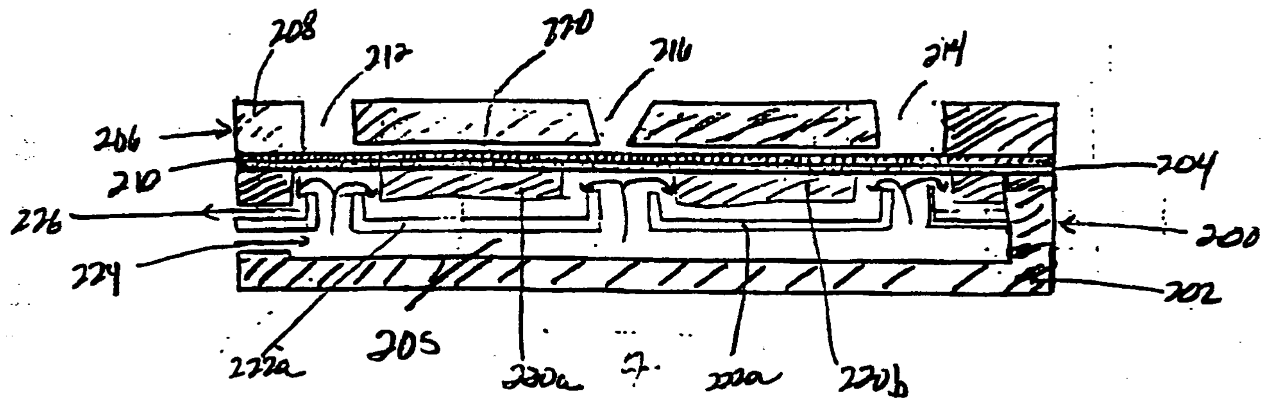
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Fig. 7

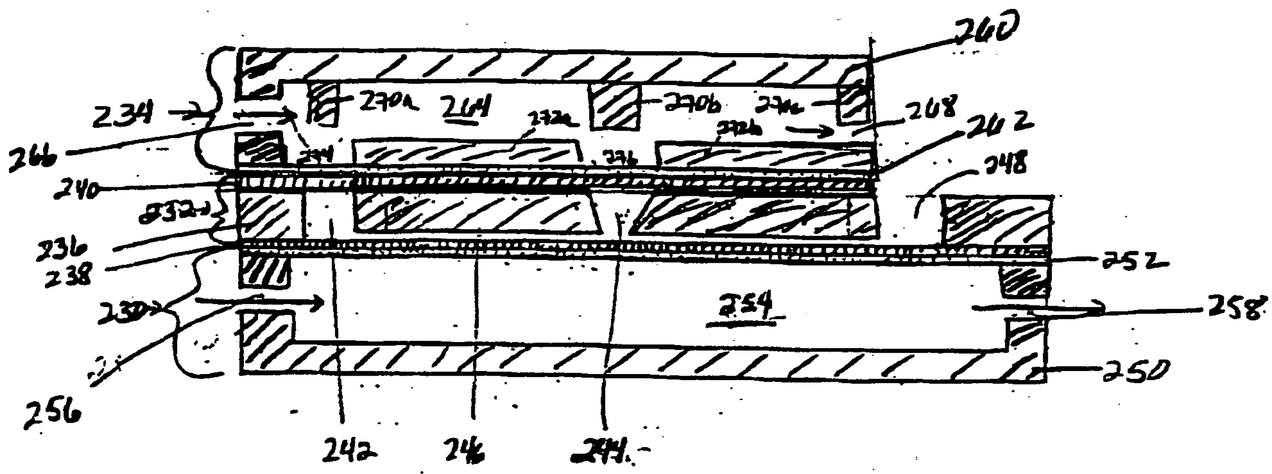
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Fig. 8

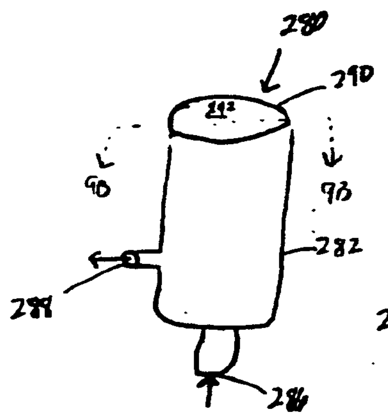

Fig. 9A

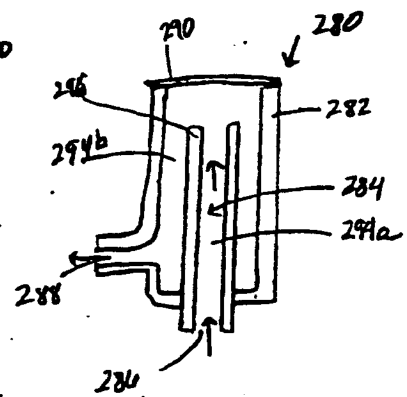

Fig. 9B

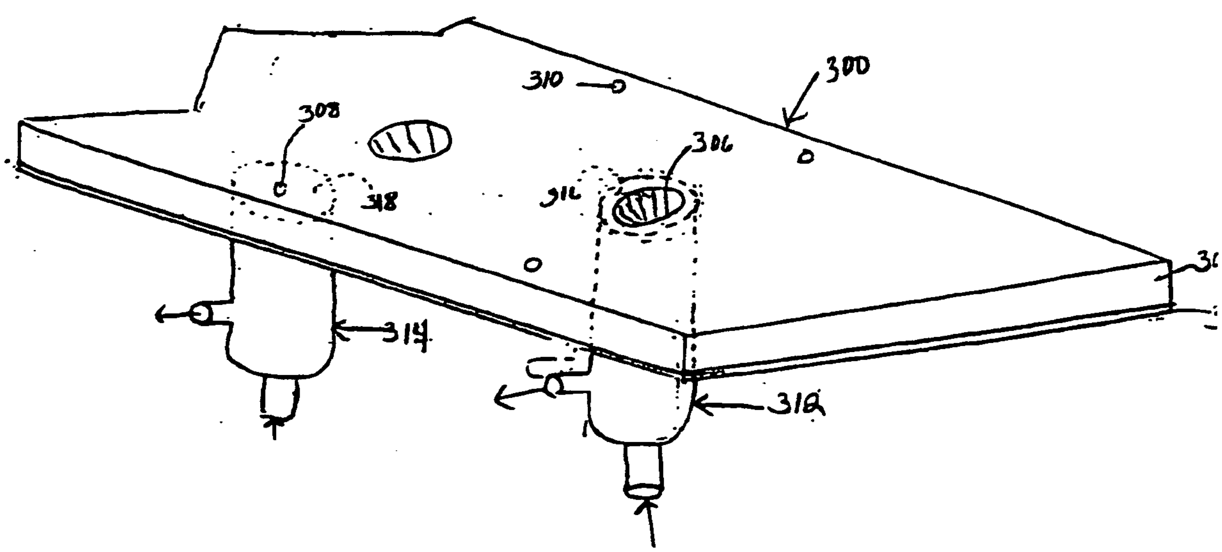

Fig. 9C

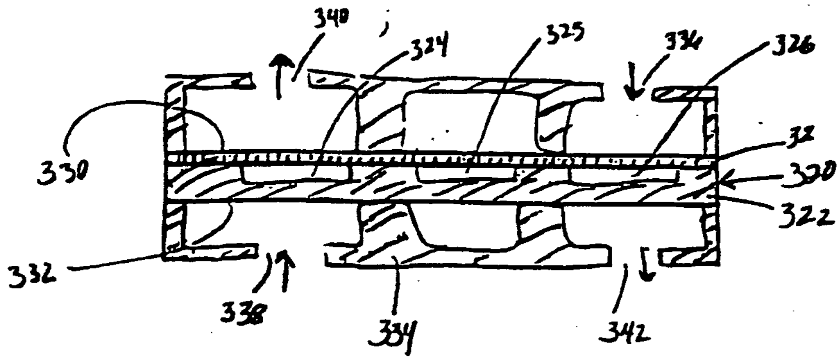

Fig. 10

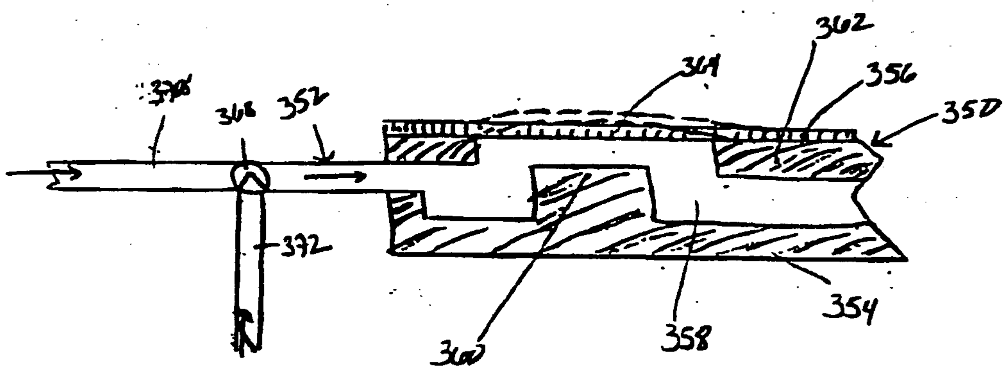

Fig. 11A

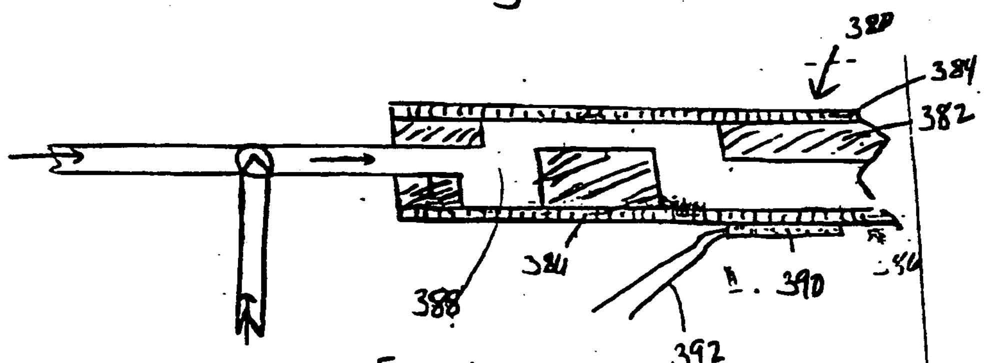

Fig. 11B

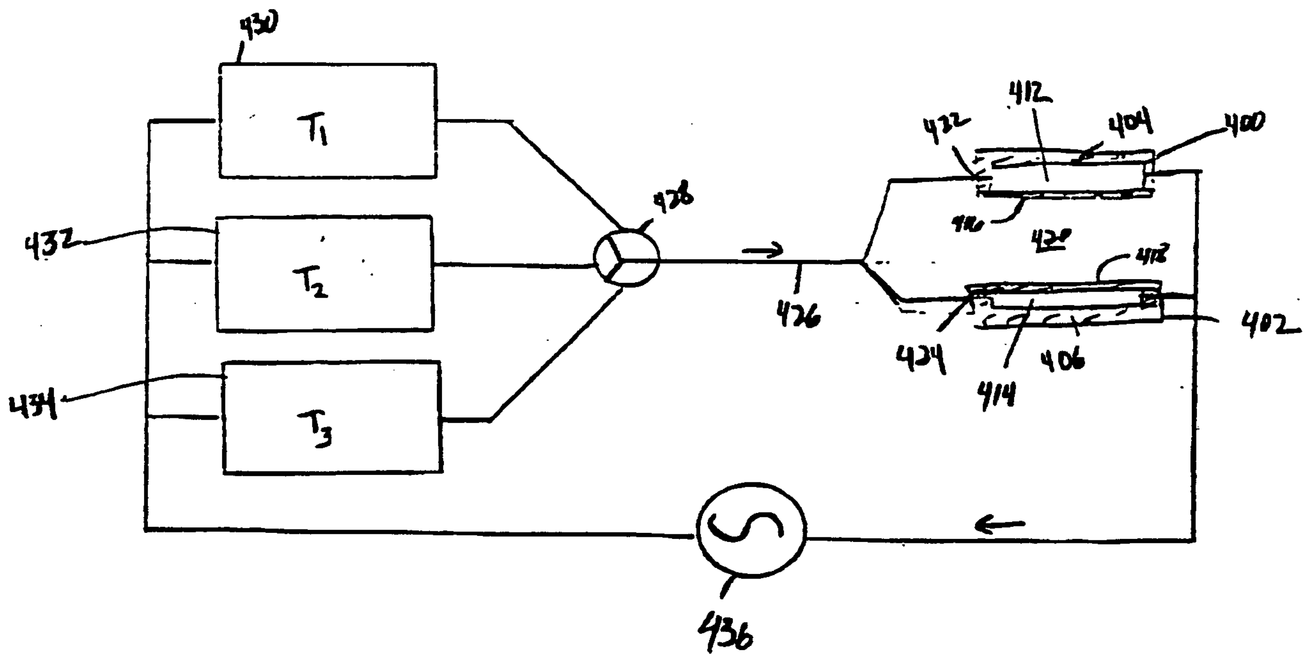
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Fig. 12

INTERNATIONAL SEARCH REPORT

International Application No
PC./US 00/28826

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/00 G01N27/26 G01N31/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 965 410 A (CHOW CALVIN Y H ET AL) 12 October 1999 (1999-10-12) claims; figures	1,12,17
A		2-11, 13-16,18

Y	WO 98 50147 A (UNIV CALIFORNIA) 12 November 1998 (1998-11-12) cited in the application claims; figures	1,12,17
A		2-11, 13-16,18

A,P	WO 99 64848 A (CALIPER TECHN CORP) 16 December 1999 (1999-12-16) claims; figures 1-3	1-18

[ ] Further documents are listed in the continuation of box C.

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22 December 2000	27.03.01

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/28826

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5965410 A	12-10-1999	AU 9210398 A	22-03-1999
		EP 1009995 A	21-06-2000
		WO 9912016 A	11-03-1999
		US 6174675 B	16-01-2001
WO 9850147 A	12-11-1998	AU 7477398 A	27-11-1998
WO 9964848 A	16-12-1999	AU 4427399 A	30-12-1999